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Is F_2-isoprostane a biological marker for the early onset of type 2 diabetes mellitus?

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Aim: This clinical study was undertaken to investigate whether F_2-isoprostane, a prostaglandin F_2-like compound derived from the non-enzymatic peroxidation of arachidonic acid, could serve as a novel biomarker for early detection of type 2 diabetes mellitus (T2DM) or its complications. **Materials and Methods:** Twenty-five individuals who had impaired glucose tolerance (IGT) or T2DM were compared with controls. Anthropometry, plasma glucose, hemoglobin A1c, lipid profile and F_2-isoprostane levels were measured in these subjects. **Results:** Clinical characteristics of subjects with IGT showed significant alteration when compared to subjects with T2DM but not with that of controls having normal glucose tolerance. In the case of isoprostane levels, there existed a significant difference between groups but there is no clear correlation with the clinical characteristics of the subjects. **Conclusion:** Isoprostane may not serve as a marker for early detection of diabetes. However, its value may predict the oxidative status of the subjects and hence the development of the complications associated with diabetes.

KEY WORDS: Impaired glucose tolerance, isoprostanes, normal glucose tolerance, type 2 diabetes mellitus

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Introduction

Diabetes is one of the most common non-communicable diseases globally. It is either the fourth or fifth leading cause of death in most developed countries. It is one of the most challenging health problems faced by many developing and industrialized countries.[1] The cause of type 2 diabetes mellitus (T2DM) is insulin resistance and relative insulinopenia.[2] One of the foremost challenges we face is to account mechanistically for the myriad other biochemical and physiological abnormalities characteristic of this disease. These abnormalities include central obesity, hypertension, accelerated atherosclerosis, hypertriglyceridemia and low serum concentrations of high density lipoproteins.[3,4]

Isoprostanes, a new class of biologically active products of arachidonic acid metabolism, are emerging as potentially relevant to human vascular disease. Besides providing a likely index of lipid peroxidation in this setting, measurements of specific F_2-isoprostanes in urine may provide a sensitive biochemical end-point for dose-finding studies of natural and synthetic inhibitors of lipid peroxidation. F_2-isoprostanes are prostaglandin like compounds formed *in vivo* from free radical catalyzed peroxidation of arachidonic acid, via a non-cyclooxygenase dependent mechanism. F_2-isoprostanes are found in the body tissues in the esterified form, and in biological fluids such as plasma and urine in the free form.[5] Enhanced formation of F_2-isoprostanes has been associated with several cardiovascular risk factors including hypertriglyceridemia[6] and diabetes mellitus[7] that are characterized by increasing lipid peroxidation and in response to other abnormalities such as atherosclerosis.[8] Among the 15 series of isoprostanes, F_2-isoprostanes possess more marked biological functions such as vasoconstriction, stimulating mitogenesis, enhancing monocytes and polymorphonuclear cell adhesion to endothelial cells and inducing endothelial cell necrosis. This led us to focus our interest on F_2-isoprostanes rather than other isoprostanes.

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Impaired glucose tolerance (IGT) which can be assessed by oral glucose tolerance test is widely accepted as an entity of the prediabetic stage. It is commonly believed that IGT represents a transitional stage between normal glucose tolerance (NGT) and diabetes. Moreover, IGT is also a risk factor for cardiovascular disease either by itself or as a consequence of its presumed precursor relation to diabetes.[9] However, IGT is not a homogenous condition and only some of the subjects in transitional stage reach frank diabetes. Therefore, it is important to identify a more reliable marker for identifying people at high risk for diabetic complications.

This study was undertaken to (a) investigate the usefulness of F_2-isoprostane as an oxidative biomarker in T2DM, (b) estimate the levels of F_2-isoprostane among diabetics and (c) measure F_2-isoprostane levels in IGT subjects as an early marker. The study was conducted among three groups of subjects: diabetic, NGT and IGT.

Materials and Methods

Subjects

The study included 25 diabetic, 25 NGT and 25 IGT subjects. The diabetics were those who have been diagnosed earlier and are currently on medication. The subjects were classified as having NGT if their fasting plasma glucose levels were less than 6 mmol/L and the 2-hour plasma glucose loads values were less than 7.8 mmol/L.[10]

Anthropometric measurements

Anthropometric measurements like weight and height were obtained using standardized techniques as detailed elsewhere.[11] Height was measured with a tape to the nearest centimeter. Weight was measured with a traditional spring balance that was kept on a firm horizontal surface. The body mass index (BMI) was calculated from the weight and height.

Laboratory studies

After an overnight fast, venous blood samples were drawn from each patient in sterile ethylenediamine tetraacetic acid (EDTA) tubes. Samples were assayed for plasma glucose, HbA1c, total cholesterol, triglycerides and high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol.

Measurement of isoprostane

Five hundred microliters of serum was acidified to pH 3.5 with 100 µL of hydrochloric acid (12.1 M) and allowed to equilibrate at 4°C for 15 minutes. It was then centrifuged at 14,000 rpm for 5 minutes and applied to C18 cartridges (IST, Mid Glamorgan, UK) which had been preconditioned with 5 mL of ethanol and 5 mL of deionized water. The cartridges were subsequently washed with 5 mL of 15% ethanol and hexane (Merck, San Diego, CA, USA) under slight positive pressure to obtain a flow rate of 0.5 mL/minute. Isoprostanes were eluted from the column with 5 mL of ethyl acetate (Schaelau, Barcelona, Spain) and stored at –70°C. Elutes were then evaporated under a stream of nitrogen and reconstituted in 230 µL of assay buffer. F_2-isoprostane in elute was quantified using competitive immunoassay according to the manufacturer's instructions (Assay Design, Ann Arbor, MI, USA). F_2-isoprostane standards included in the kit were used within 60 minutes of preparation. Elutes and standards were pipetted into a microtiter plate coated with goat anti-rabbit immunoglobulins. Exactly 50 µL of alkaline phosphate conjugated with F_2-isoprostane antibody (rabbit polyclonal antibody to F_2-isoprostane) was added and incubated at room temperature on a shaker for 2 hours at 500 rpm. Wells were washed twice with the assay buffer before the substrate (*P*-nitro phenyl phosphate) was added and incubated further for 45 minutes without shaking. Finally, enzyme reactions were stopped by the addition of 50 µL of trisodium phosphate and the optical density was read at 405 nm using a microplate reader.

Statistical analysis

Experimental data were expressed as mean ± SD. Statistical analysis was performed by using non-parametric (Kruskal-Wallis) one-way analysis of variance (ANOVA) followed by Dunn's test. A *P* value of less than 0.0001 was considered statistically significant between the groups. Statistical calculations were carried out using Graphpad Prism, version 5.0 (Graph Pad Software Inc., Los Angeles, San Diego, California, USA).

Results

A total of 75 subjects, grouped according to those with NGT (control, n = 25), IGT (n = 25) and established diabetes (T2DM, n = 25) were studied. The clinical and biochemical profiles of the study subjects are shown in Table 1. There were no significant differences between the groups IGT and NGT, but age, BMI, fasting plasma glucose, HbA1c, total cholesterol, triglycerides, LDL cholesterol were significantly higher among subjects with T2DM compared to subjects with NGT and IGT (P < 0.0001). Plasma concentration of total F_2-isoprostane in subjects with T2DM and IGT had significantly

Table 1: Clinical characteristics of study subjects

	NGT (n = 25)	IGT (n = 25)	T2DM (n = 25)
Age, years	35.8 ± 3.41	39 ± 5.02	58 ± 5.77**#
BMI, kg/m^2	24.0 ± 2.35	27.3 ± 2.58	28.6 ± 4.94**
Fasting plasma glucose (mmol/L)	4.76 ± 0.572	5.29 ± 0.807	11.0 ± 1.37**#
HbA1c (%)	5.53 ± 0.337	6.03 ± 0.687	11.0 ± 0.67**#
Total cholesterol (mmol/L)	4.81 ± 0.55	4.93 ± 0.72	7.19 ± 0.56**#
Triglyceride (mmol/L)	1.79 ± 0.53	1.95 ± 0.64	4.13 ± 0.57**#
HDL cholesterol (mmol/L)	1.87 ± 0.26	1.78 ± 0.31	1.03 ± 0.13**#
LDL cholesterol (mmol/L)	2.63 ± 0.51	2.76 ± 0.65	3.99 ± 0.63**#

Data are expressed as mean ± SD; **P < 0.0001 as compared to NGT; #P < 0.0001 as compared to IGT

higher mean values compared with that of NGT group (P < 0.0001) [Figure 1].

Discussion

This study evaluates whether F_2-isoprostane is an early marker for development of diabetes or IGT. IGT is the intermediate state which exists between NGT and the overt diabetes.[11-15] Insulin resistance and impaired β-cell function are the primary defects observed in T2DM, which can be detected in subjects with IGT. However, clinical studies suggest that the site of insulin resistance varies between these two disorders. Subjects with IGT have marked muscle insulin resistance with only mild hepatic insulin resistance. Impairment of insulin secretion in individuals with IGT remains controversial because both lower and normal early and late insulin secretory responses have been reported.[15-17] Likewise, in our study, patients with IGT had lower fasting glucose levels than patients with T2DM

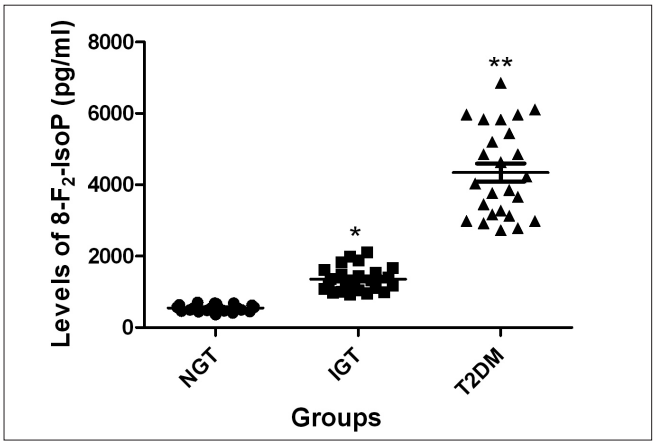

Figure 1: Plasma F_2-isoprostane levels among subjects with NGT, IGT and T2DM

suggesting the late-phase of onset of diabetes. This study is of significance as this is the first report in Malaysian population on the association of F_2-isoprostane levels with IGT, particularly in prediabetic subjects. Il'yasova *et al*,[18] showed in a case–control study that there exists no longitudinal association of isoprostanes levels [as measured by gas chromatography–mass spectrometry (GC–MS)] in 26 cases of incident T2DM, but they did not investigate subjects with IGT. It may be that isoprostanes are biologically insufficient to independently induce hyperglycemia, or alternatively, a larger sample that may have more power to discriminate between the glycemic groups may be needed. A negative correlation between F_2-isoprostanes, clinical characteristics and the progression to IGT or T2DM implies that F_2-isoprostanes might be involved in the development of complications of diabetes but not in the onset. Unlike other reports, isoprostanes in our population did not serve as valuable markers for the early onset of diabetes.

In conclusion, our findings do not support the hypothesis that isoprostane is a good marker for early onset of diabetes. Further studies on genetic profile of these populations may reveal a clearer delineation. However, F_2-isoprostanes may be good markers for the development of cardiovascular complications of diabetes[4] and are correlated with hypercholesterolemia.[5]

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